Critical functions of Reck in mouse forebrain development(Abstract_要旨)

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（続紙 1 ）

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論文題目	Critical functions of *Reck* in mouse forebrain development （マウス前脳発生における *Reck* の重要な役割）		

（論文内容の要旨）

Proper development and functioning of the brain is dependent on the reciprocal regulation between the neural and vascular systems, which comprise what is known as neurovascular unit (NVU); it is likely that several molecules expressed by neuroepithelium, vascular cells, of both contribute to the crosstalk between these two systems. Previously, RECK in neural precursor cells (NPCs) was found to support Notch-dependent neurogenesis. Recent studies implicate RECK expressed in endothelial cells (ECs) in WNT7-induced canonical WNT signaling required for angiogenesis in the central nervous system (CNS). The applicant reports that selective inactivation of *Reck* in Foxg1-positive NPCs in mice results in an unexpected phenotype characterized by the death shortly after birth with severe hemorrhage in the forebrain. As expected, these mice also show a defect in neurogenesis characterized by precocious neuronal differentiation. The hemorrhage, on the other hand, is accompanied by vascular malformations that are similar (in morphology, location, and timing) to those found in EC-specific *Reck* knockout mice as well as Wnt7a/7b double knockout mice. Notably, the hemorrhage could be partially rescued by administration of LiCl, an activator of WNT signaling, into the parental pregnant female mice, suggesting that impairment of canonical WNT signaling is involved in this phenotype. The *Reck*-deficient NPCs showed reduced potency to active canonical WNT signaling when co-cultured with the WNT reporter (TOP-Flash) cells *in vitro*. In a model system using the transfectable HEK293 cell line, an activity of RECK in WNT7-producing cells to enhance canonical WNT signaling in reporter cells was detectable in co-culture assay but not in the experiments using conditioned media. These findings indicate that RECK in NPCs has a non-cell-autonomous function to promote forebrain angiogenesis, probably through contact-dependent enhancement of WNT signaling in ECs, and provide a fresh insights into the functions of RECK and its role in brain development.

（続紙 ２ ）

（論文審査の結果の要旨）

During embryonic development, reciprocal communications between neural and endothelial cells (EC) are not only the basis of the neurovascular unit but also contribute to the formation and maturation of blood-brain barrier. Molecular mechanisms of such communications remain largely elusive. A previous study on the phenotype of global *Reck*-knockout mice revealed the role of RECK in neural precursor cells (NPCs) to support Notch-dependent neurogenesis. On the other hand, recent studies by several groups have revealed that RECK in ECs plays a role in WNT7-induced canonical WNT signaling known to be critical for angiogenesis in the central nervous system (CNS).

The applicant Huiping Li attempted to confirm and extend the neural function of RECK by generating NPC-selective conditional knockout mice [Reck-cKO (Foxg1)]. Unexpectedly, these mice die shortly after birth with hemorrhage in the forebrain. To understand how *Reck* in NPCs could regulate angiogenesis, the applicant compared the characteristics of the hemorrhage in [Reck-cKO (Foxg1)] and EC-selective *Reck* cKO mice [Reck-cKO (Tie2)] and found that the hemorrhage was accompanied by vascular malformations very similar to those found in Reck-cKO (Tie2) mice and in *Wnt7a/7b* double knockout mice. The hemorrhage could be partially rescued by administration of LiCl, an activator of WNT signaling, to the parental mice. Furthermore, two endogenous targets of canonical WNT signaling (*Apcdd1* and *Sox17*) were downregulated in the mutant forebrain, supporting the idea that canonical WNT signaling is attenuated in the forebrain of Reck-cKO (Foxg1) mice. This idea was also consistent with his data indicating that NPCs derived from forebrain could activate WNT signaling in adjacent cells, as assessed by TOP-Flash assay, in a contact-dependent manner and that such activity was attenuated in NPCs derived from Reck-cKO (Foxg1) mice. These findings raise the possibility that RECK in NPCs has a non-cell-autonomous function to promote forebrain angiogenesis, probably through contact-dependent enhancement of WNT signaling in ECs.

This study provides fresh insights into the functions of RECK in brain development, how paracrine WNT signaling can be facilitated, and how neuro-vascular communication during brain development can be achieved.

The thesis defense was held on September 3, 2019. The applicant presented his findings in a clear and logical way and showed his knowledge, ability, and integrity as a scholar through the discussion. The committee therefore admitted that the thesis and applicant satisfy the requirements for the degree of Doctor of Philosophy in Life Sciences, Kyoto University.

論文内容の要旨及び審査の結果の要旨は、本学学術情報リポジトリに掲載し、公表とする。特許申請、雑誌掲載等の関係により、学位授与後即日公表することに支障がある場合は、以下に公表可能とする日付を記入すること。 **（ただし、学位規則第８条の規定により、猶予期間は学位授与日から３ヶ月以内を記入すること。）**

要旨公開可能日：　　年　　月　　日